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A Mini-Review on Smut Disease of Sugarcane Caused by *Sporisorium scitamineum*

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1. Introduction

Smut of sugarcane is caused by the fungus *Ustilago scitmainea* (Sydow, 1924). The first report of the disease incidence came from Natal, South Africa in 1877 as reported by Luthra *et al*., (1940) and it was speculated to be confined in the eastern hemisphere, until it was reported in Argentina. The disease spread is worldwide covering most of the sugarcane producing areas *viz*. Mauritius, Rhodesia, Indonesia, the islands of Java, Sulawesi and Sumbawa, etc. Until the 1950s, smut was of concern only in Asia, with an outlying population in Argentina. Since then, it has spread through South, Central, East and West Africa, where many of the areas not having focused breeding programme for smut resistance. In the 1970s and 1980s, it expanded to Hawaii, the Caribbean, the mainland USA, Central America and Southern Brazil. These outbreaks prompted a great deal of experimental work on sugarcane smut (Heinz, 1987). Subsequently, the occurrence of sugarcane smut in Morocco (Akalach, 1994) and Iran (Banihashemi, 1995) was established. The occurrence, prevalence and importance of the sugarcane smut pathogen have been highlighted by Antoine as early as 1961. The incidence of the disease was widespread covering several countries in East Africa, the Pacific and the Caribbean islands, wherein a severe outbreak of the disease resulted in devastating loss to the sugarcane plantations. Lovick (1978) comprehensively reviewed on various aspects of sugarcane smut *viz*. symptoms, yield reduction, causal organism, physiological races of the smut fungus, epidemiology, host resistance and management. It was reported that severe smut outbreak in the Caribbean has created an impact amongst cane growers and sugar industry.

The most recognizable diagnostic feature of sugarcane infected with smut is the emergence of a long, elongated whip. The whip morphology differs from short to long, twisted, multiple whips etc. (Fig.1). Affected sugarcane plants may tiller profusely with spindly and more erect shoots with small narrow leaves (i.e., the cane appears ''grass-like'') with poor cane formation (Fig.2). Others symptoms are leaf and stem galls, and bud proliferation (Fig.3). The disease can cause significant losses in cane tonnage and juice quality; its development and severity depend on the environmental conditions and the resistance of the sugarcane varieties. Successful management of smut in sugarcane relies more on exploiting host resistance. To enhance smut resistance in commercial hybrids, intensive breeding programs should be formulated by involving exotic clones as source of resistance from germplasm exchange. In this connection, identifying resistance genes in the wild *Saccharum spontaneum* and bringing up of newer resistant clones along with an efficient screening

program could significantly reduce the disease incidence. Singh *et al*., (2005) concluded that ITS-based probing could not bring out much of variability among *S. scitamineum* isolates in South Africa and suggested that IGS-based studies could possibly discriminate genetic variability amongst the isolates representing different sugarcane growing regions of the world. Raboin *et al*., (2007) hypothesized that in sugarcane smut, pathogenic variability is greater in Asian countries, where a high level of genetic variation in *S. scitamineum* is reported. With the advent of more precise molecular tools, it is now possible to understand better - pathogen variability *vis a vis* host resistance, that would augment well for successful disease management in the future.

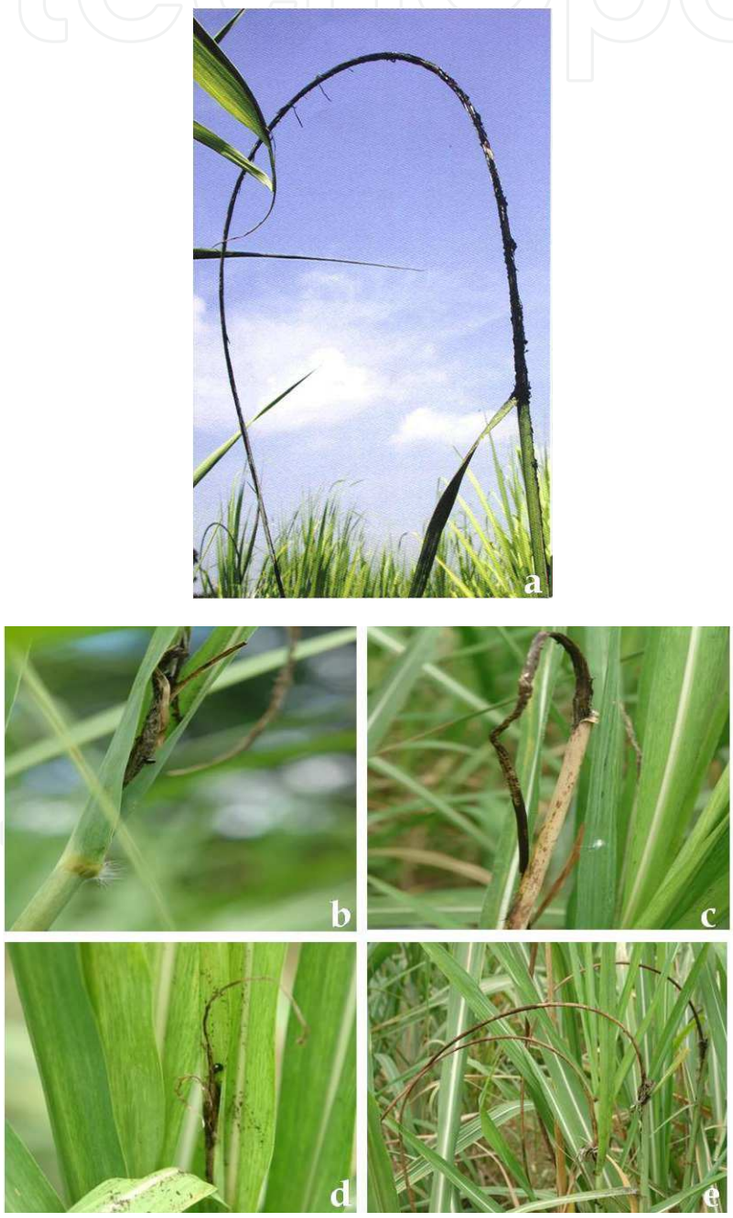

Fig. 1. **Different forms of whip morphology in smut infected sugarcane.** a) Long whip. b) Closed whip. c) Twisted whip. d) Short whip. e) Multiple whips.

Fig. 2. **Smut infected clump.** Characteristic symptoms of profuse tillering and poor cane formation (left) as compared to healthy canes (right).

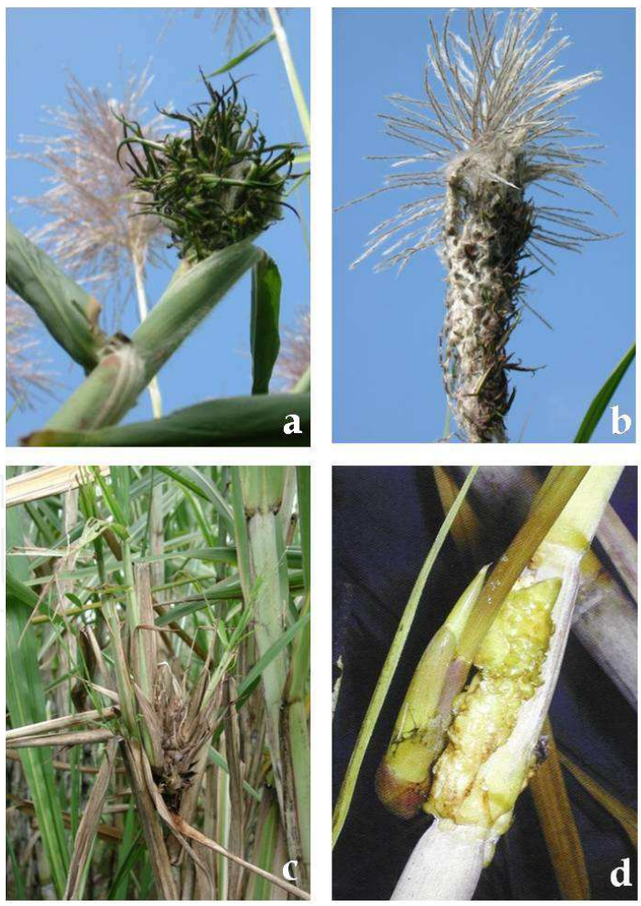

Fig. 3. **Unusual symptoms due to smut infection.** a) Apical deformity. b) Floral infection. c) Malformed spindle. d) Bud proliferation.

2. Distribution

Smut disease of sugarcane can cause considerable yield losses and reduction in cane quality (Ferreira & Comstock, 1989). The disease is sometimes referred to as "culmicolous" smut of sugarcane, because it affects the stalk of the cane. Smut disease resulted in significant yield losses in sugarcane production and was reported to be distributed all over the sugarcane growing areas in China (Huang *et al*., 2004). At one time or another, sugarcane smut has been important in nearly every sugarcane growing country in the world. Australia is a major exception, since the disease was initially present only in Western Australia, a minor production area. The disease was first reported in Australia in the Ord River Irrigation Area (ORIA) in 1998. The most likely source of this infection was thought to be windblown spores from Indonesia (Riley & Jubb, 1999). Parts of eastern Australia, Fiji and Papua New Guinea were reported to be still free from the disease (Braithwaite *et al*., 2004). The growing significance of this pathogen is clearly evident with the flowing research papers on various aspects of the disease *viz*. host resistance, pathogen variability and diagnosis, management etc., during the past decade. Antony (2008) comprehensively reviewed the status of sugarcane smut in Australia and discussed about the political economy of biosecurity incited due to severity of the disease. More than 70% of Australia's sugarcane varieties were susceptible to smut before 1998. It was not possible to completely eradicate the disease by the time smut was noticed, as it has spread in the whole area under sugarcane cultivation. The immediate management strategy devised was to advocate ploughing out canes with more than 5% infection and switching over to resistant varieties. Since then, smut resistance became an objective of varietal selection in Australia (Croft & Berding, 2005).

3. Epidemiology

A detailed epidemiological study on sugarcane smut was made by Bergamin *et al*., (1989), who recorded alarming proportions of smut in Brazil. The increase in incidence was found to be associated with varietal susceptibility and increasing age of the crop. The first appearance of the apical whips was found to coincide with around 120 days of planting. The second flush of whip emergence produces an enormous quantity of teliospores and these account for infecting the terminal and lateral buds in the rapidly growing crop. The infected buds may remain dormant and may germinate to produce lateral whips in the third flush of whip production. The infection producing the third level of whips is believed to be critical in the epidemiology of the disease.

4. Pathogen

Germination of smut spores occur on the internodal surface (Fig. 4), which was followed by the formation of appressoria on the inner scales of the young buds and on the base of the emerging leaves. Entry into the bud meristem occurs between 6 and 36 h after the teliospore deposition (Alexander & Ramakrishnan, 1980). Hyphae are found throughout the plant mostly in the parenchymatous cells towards the lower internodes. In the upper internodes, the hyphae are progressively built up culminating in the formation of whip (sori with teliospores). Infective mycelia penetrate through the buds at each node and systemically colonize the apical meristem. Infective buds in mature plants are either symptomatic as whip at the end of stalk or remain asymptomatic hidden in buds up to the next season (Agnihotri, 1990).

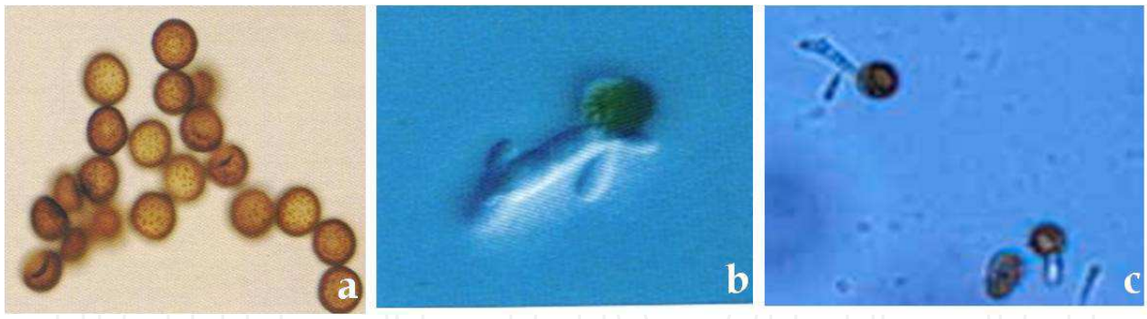

Fig. 4. **Smut teliospores and its germination.** a) Teliospores from whip. b) & c) germination of teliospores.

Sexuality has been demonstrated in the smut pathogen by Alexander and Srinivasan (1966), who showed that it was bipolar, that a combination of two sporidia belonging to opposite sexes was necessary for successful infection and the degree of virulence varied with the combination of haplonts. The existence of physiological specialization has been demonstrated by Alexander & Padmanaban, (1992) and Amire *et al*., (1982). Classification of races of *U. scitaminea* is based on differences in spore morphology, germination characteristics or pathogenic nature (Sydow,1924). Similar to other species of *Ustilago*, the sugarcane smut fungus is a parasite of young meristematic tissues and gains entry into the host, exclusively through the bud scales (Fawcett, 1942). The pathogen develops systemically throughout the stalk, but teliospores are formed only in peripheral tissues of the whip-like structure. The fungus is capable of mutating and hybridizing in nature in order to produce new virulent pathogenic races (Waller, 1970).

Piepenbring *et al*., (2002) regrouped the generic position of the sugarcane smut pathogen and renamed it as *Sporisoriun scitamineum*. Three species of smut fungi (Ustilaginales, Basidiomycota) of economic importance, *Ustilago maydis* on corn, *U. scitaminea* on sugar cane, and *U. esculenta* on *Zizania latifolia*, were investigated in order to define their systematic position using morphological characteristics of the sori, ultrastructure of teliospore walls, and molecular data of the LSU rDNA. LSU rDNA analysis suggested that *U. scitaminea* belong to the genus *Sporisorium*. The sugarcane smut fungus develops sori with whip-shaped axes corresponding to columellae and henceforthe, *U. scitaminea* is called *Sporisorium scitamineum*.

5. Variability

Information on the prevalence and distribution pattern of races/pathotypes in *S. scitamineum* in an area is required for effective deployment of host resistance. Schenck (2003) recorded incidence of smut in one variety (H78-7750), considered to be completely resistant in several seed fields on Maui, indicating the possible emergence of a new race of the smut fungus in Hawai. The new smut race was included in breeding program susceptibility screening, keeping in mind, that smut resistant varieties should also be treated and monitored even though the appearance of new smut races was presumed to be quite rare. The use of differential hosts is a viable option for the evaluation of pathogenic variability. However, not much of information on the use of differential hosts is available in sugarcane against the smut pathogen. Gillaspie *et al*., (1983) used seven sugarcane clones

(*Saccharum* interspecific hybrids) for inoculation with *S. scitamineum* isolates collected from Argentina, Florida, Hawaii, Taiwan, and Zimbabwe. Six different isolates (races) could be differentiated on five of the clones under greenhouse conditions and it was concluded that this method is a valid, rapid method for isolate separation when the correct differential clones are used. It was also observed that the environment effects on the teliospores might be confounded with genetic differences amongst the test isolates which might probably complicate breeding for smut resistance. Smut pathogen being biotrophic, the inoculum henceforth was to be maintained in the standing cane as teliospores, however the fungus has been successfully cultured in an artificial medium in the recent past. Slow growing fluffy white mycelia was observed from actively growing meristem tips cultured under aseptic conditions (Fig. 5), which was further used for molecular characterization of pathogen variability. Smut isolate collection is made from different representative sugarcane growing areas in India and the pathogen variability is being investigated using differential hosts and molecular markers *viz*. RAPD, SSR etc (Ramesh Sundar *et al*., 2011 - personal communication).

The 20th century saw the steady spread of sugarcane smut to almost all sugar industries of the world (reviewed by Presley, 1978). A widely adapted, stable smut pathotype may have been involved in this spread, explaining the lack of genetic variation in isolates collected from countries outside of Asia. Pathogenic races of sugarcane smut have been observed in several countries including two races (A and B) from Hawaii (Comstock & Heinz, 1977) and three races (1, 2, 3) reported in Taiwan (Leu *et a.l*, 1976). However, Ferreira and Comstock (1989) considered the true prevalence of races to be controversial. Many claims were based on the reaction of the same cultivar in different countries, but the interpretation of these claims was confused by test-to-test variation and the use of different inoculation methods in different countries.

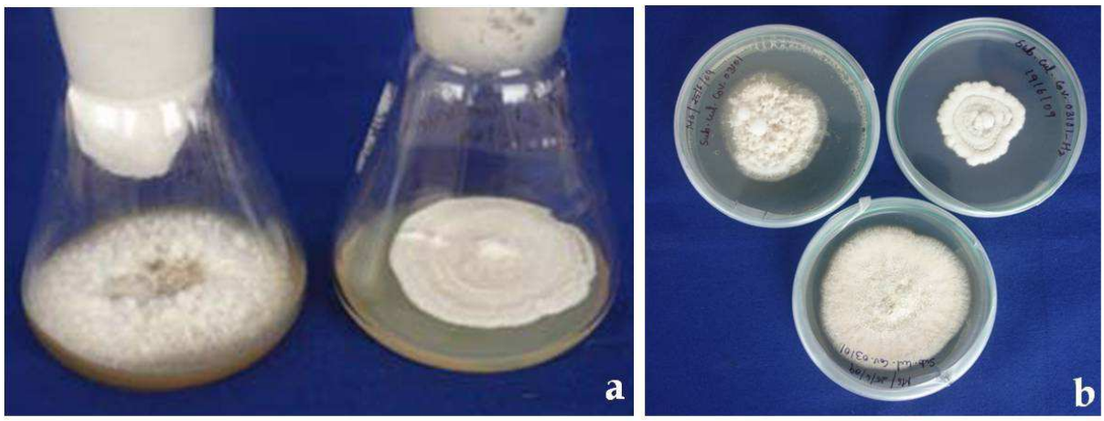

Fig. 5. ***In vitro* culture of smut dikaryotic mycelium.** a) & b) depicts existing morphological variations amongst isolates.

DNA-based markers have been known to detect and measure the variability among individuals and work on molecular characterisation of smut pathogen variability is being carried in many laboratories worldwide in the recent past. Combined application of molecular diagnostic tools along with use of differentials could be an appropriate and reliable approach for studying pathogen variability in *S. scitaminieum*. Braithwaite *et al*., (2004) employed amplified fragment length polymorphisms (AFLPs) to assess genetic

variation between 38 isolates of the sugarcane smut fungus representing 13 countries. The study identified a divergent group of isolates from Southeast Asia. *S. scitamineum* is phenotypically variable with regard to morphology, cultural characteristics and pathogenicity (Abo & Okusanya, 1996). These phenotypic differences appear to be greater than the genetic differences as detected by the neutral AFLP markers, suggesting that these phenotypes correlate with minor changes in the genome or possibly in single genes. They could also be indicative of environmental differences and/or gene expression differences. The results further suggested that alternative fingerprinting techniques, such as simple sequence repeats (SSRs or microsatellites), might provide higher sensitivity and generate more polymorphisms to reveal the existence of yet other clusters.

Xu *et al*., (2004) studied the genetic diversity of sugarcane smut fungus representing different provinces in Mainland China applying RAPD. Dendrogram of UPGMA cluster analysis revealed that 18 isolates of the fungus were clustered into six groups according to the dissimilarity coefficient of 0.70. The results of cluster analysis suggested that the molecular variation and differentiation could be associated with geographical origin to some extent, but not applicable to all isolates. It might be due to the frequent exchange of sugarcane varieties and clones in the recent years. Molecular diversity analysis observed no relationship between pathogen variability and host origin.

Singh *et al*., (2005) estimated intraspecies diversity within *Ustilago scitaminea* isolates from South Africa (SA), Reunion Island, Hawaii and Guadeloupe using RAPDs, *b*E mating-type gene detection, rDNA sequence analysis, and spore morphological studies. Mycelial DNA of the South African isolate shared 100% sequence identity with that of mycelial DNA cultured from *in vitro* produced teliospores of the parent cultivar. Overall the ITS1 and ITS2 regions were found to have 96.1% and 96.9% sequence identity with a total of 17 and 21 base changes, respectively, amongst the isolates. The Reunion Island isolate was shown to be most distantly related by 3.6% to the other isolates, indicating a single clonal lineage. The lack of germination in teliospores from Guadeloupe might be attributed to changes in temperature and humidity during transportation.

Raboin *et al*., (2007) investigated the genetic diversity and structure of different populations of the smut fungus worldwide using microsatellites by subjecting 77 distinct whips (sori) collected in 15 countries worldwide. Results indicated that the genetic diversity of either American or African *S. scitamineum* populations was found to be extremely low and all strains belonged to a single lineage. This lineage was also found in some populations of Asia, where most *S. scitamineum* genetic diversity was detected, suggesting that this fungal species originated from this region. The results obtained in this study thus suggested that the use of resistant cultivars to *S. scitamineum* might be an efficient and durable strategy to control sugarcane smut outside Asia.

Comstock et al., (2007) comprehensively reviewed the status of genetic diversity in S.scitamineum and summarized in line with the results presented during the International Sugarcane Technologists workshop 2006. It was concluded, that the fungus originated in Asia and was disseminated to other continents on rare occasions. It was also indicated that, the resistance reaction of sugarcane clones tested in various countries was strongly influenced by the environment. The possibility of using Near Infra Red spectroscopy (NIR)

in prediction of disease resistance rating for smut disease was investigated. The results were promising and the model provided acceptable predicted ratings for all the clones.

Munkacsi *et al*., (2007) suggested that domestication and cultivation of crop plants did not drive divergence and speciation of smut species on maize, sorghum, and sugarcane. The results obtained greatly weakened a hypothesis, that the speciation of crop pathogens is the necessary result of agricultural practices, and further, showed that these fungi diverged in natural populations of the fungus and host. Most importantly, the findings demonstrated that the domestication process very likely retained symbioses between the crops and scores of microbes, which had co-evolved in ancestral, natural populations. Fattah *et al*., (2009) attempted genotyping of the races of *Ustilago* species in Egypt using the chitinase gene primers. The study concluded that chitinase genes are the most suitable for genotyping study between sugarcane smut fungal isolates. The results obtained by differential display techniques showed that there were at least 10 different races from the *Ustilago sp*. in Egyptian field. Nzioki *et al*., (2010) attempted to identify presence of physiological races of sugarcane smut and the results suggested possible existence of smut races in Kenya.

6. Diagnosis

Correct diagnosis of pathogens is the primary requirement in any sound disease management practice. It is important for the identification of pathogens, breeding crops for resistance to pathogens and epidemiological studies. Conventional approaches involve use of microscopy combined with specific stains for histopathological studies. Serology-based diagnostic techniques proved to be equally efficient in the diagnosis of sugarcane pathogens. Sinha and Singh (1982) developed a staining technique using trypan blue for the detection of smut hyphae in nodal buds of sugarcane (Fig. 6). This rapid staining technique enabled detection of hyphae of *S. scitamineum* in the growing points of nodal buds of sugarcane. The results concluded that this whole detection process can be completed within

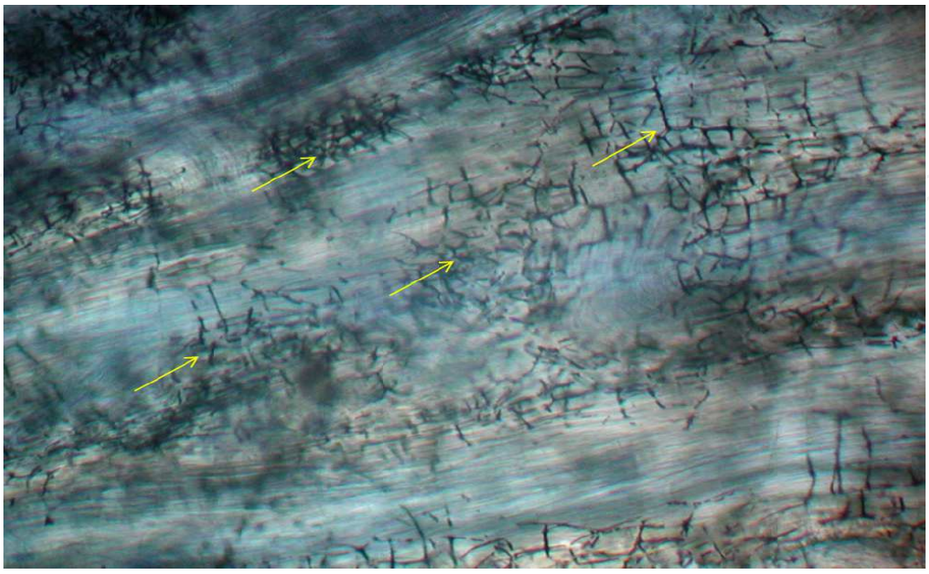

Fig. 6. **Trypan blue staining of smut fungus.** Arrows indicate proliferation of inter and intracellular mycelial growth of smut fungus in nodal buds of sugarcane

4 hour period and smut infection can be detected in buds earlier than the symptom expression on planting. This technique finds application in quarantine and seed certifying agencies for screening sugarcane seed material. Nallathambi *et al*. (1998) observed that the trypan blue staining also detected smut pathogen colonization in some clones, which escaped infection in the field. Further this staining technique was found to be very rapid, precise and allows a large number of samples to be tested in a short period. An indirect ELISA technique was standardized for screening large number of sugarcane clones for smut pathogen detection (Nallathambi *et al*., 2001). This work outlines smut antigen preparation and its appropriate dilutions for an early detection of smut pathogen at symptomless infection stage in sugarcane settlings. Acevedo and Pinon (1996) developed an indirect immunofluorescence technique for the diagnosis of *S. scitamineum* infection in sugarcane. Optimization of the methodology resulted in the best dilution of the antiserum for efficient detection of the smut pathogen.

Technological advances in PCR-based methods, such as real-time PCR, allow fast, accurate detection and quantification of plant pathogens and are now being applied to practical problems. Albert and Schenck (1996) successfully amplified *S. scitamineum* with the use of primers based on the *U. maydis b*E mating type gene. Sequence analysis of the PCR amplicon yielded around 70% homology with the *b*E in *U. maydis* and *U. hordei*. The PCR-product of 459 bp is specific to *S. scitamineum* and it has been validated successfully by many researchers. Singh *et al.* (2004) demonstrated that PCR assay was extremely sensitive in detecting the presence of the pathogen and yielded a positive response in plantlets inoculated with sporidia and observed that PCR assay was significantly better for smut detection than microscopy. Whilst the PCR assay and microscopy may be used to detect the smut pathogen in plantlets not exhibiting symptoms of infection, it was concluded that there was no relationship between the presence of the pathogen and plant resistance. Yudilay *et al*., (2004) critically evaluated different diagnostic methods *viz*. conventional, optic microscopy, serological and molecular of the sugarcane smut *Ustilago scitaminea* Syd and weighed out the advantages and disadvantages of each one of them according to sensibility, efficiency and possibilities. Jorf and Izadi (2007) isolated and purified yeasts-like and dikaryotic mycelial colonies of the sugarcane smut pathogen and concluded that PCR assay and microscopic study could be used effectively to detect the presence of smut pathogen in settlings not exhibiting symptoms of infection. The results of an investigation also revealed that PCR assay resulted in more early detection of the pathogen (Ramesh Sundar *et al*., 2011 – Personal communication).

7. Host resistance

7.1 Screening for smut resistance and its biochemical indices

Releasing disease resistant varieties has been the prime management strategy to reduce the yield loss caused by the fungal pathogens in sugarcane. Burner (1993) evaluated the smut resistance of *Saccharum* spp. *viz. S. officinarum, S. barberi, S. sinense, S. robustum, S. spontaneum, Erianthus* spp. section Ripidium, and *Saccharum* interspecific hybrids (cultivars). The study revealed that clones of *Erianthus* spp. section Ripidium were the most resistant clones and clones of *S. officinarum* and *S. robustum* were the most susceptible amongst the six taxonomic groups studied. Clones from India seem to have moderate levels of resistance, whereas those from Indonesia and Philippines were found to pick more than 50% infection

on screening. Glenn *et al*., (1998) reported that *Erianthus* spp. and other wild relatives of *S. officinarum* are being used in an intergeneric sugarcane breeding programme in an effort to increase sugarcane resistance to sugarcane mosaic virus and sugarcane smut (Burner *et al*., 1993). However, breeding for disease resistance has been complicated by the frequent emergence of new pathogenic variants, which overpower the resistant varieties, as witnessed from the withdrawal of erstwhile ruling varieties from commercial cultivation. Even now, the benefit from such varieties could not be harnessed to maximize the sugarcane productivity in many of the developing countries including India, by virtue of its extreme susceptibility to important diseases like red rot and smut.

The evaluation of varieties for smut resistance is generally similar throughout the world. The rating is done based on the percentage of infected stools. Most countries employ the 0-9 disease scale of Hutchinson (1970), but differ in their assignment of infection percentage to disease rating. In evaluating smut resistance, due considerations are to be given for the percentage of infection. Waller (1970) made a pioneering work in comparing different methods of smut inoculation. Injection inoculation may induce greater smut infection than dip inoculation and the results indicated that cultivars could respond differently to the two methods of inoculation. Screening for smut reaction typically involves a dip inoculation assay in which nodal buds are immersed briefly in a suspension of teliospores, and then planted in a greenhouse. The periodical observation of smut incidence is recorded and on the basis of cumulative final percentage of disease incidence, varieties are graded as R, MR, MS S, and HS (Alexander & Padmanaban, 1988). It is a means of pre-screening large numbers of new sugarcane genotypes for resistance to smut disease. Highly resistant and resistant cultivars by this assay can then be field tested for validation and verification. Susceptible genotypes on the other hand, can be detected early and discarded. Evaluation can take place in a greenhouse or in the field (Alexander & Padmanaban., 1992).

Singh *et al*., (2005) screened tissue-cultured plantlets of three sugarcane (*Saccharum* spp.) cultivars having a known field smut reaction for smut susceptibility and established corroboration of the *in vitro* results with that of screening under field conditions. Olweny *et al*., (2008) critically evaluated the smut inoculation techniques in sugarcane seedlings and explored the possibility of screening for smut resistance at the seedling stage. Wound paste method recorded the highest incidence of smut whip production, followed by paste, however, soaking method had the lowest incidence of smut.

Basically sugarcane smut resistance mechanism is characterized into bud resistance (infection resistance) and inner tissue resistance (colonization resistance) (Dean, 1982). It was observed by Singh and Budharaja (1964), that hyphae will not penetrate cells of the scale leaves. Hence buds tightly enclosed with the scale leaves have a better chance of escaping infection. On this basis, Waller (1970) hypothesized that varietal resistance was determined by bud morphological characteristics. Structural characterization of sugarcane buds could provide clues for classification of test clones according to its smut resistance. da Gloria *et al*., (1995) established an association between the bud structural characteristics and the cultivar resistance. Presence of outer most scales were hypothesised to provide protection against the bud invasion of the smut pathogen.

It is well documented that plants have evolved effective resistance mechanisms, that enable them to defend themselves against pathogen attack. Many reports are available on this front

involving sugarcane and the smut pathogen, which suggested a chemical resistance mechanism than the morphological one. Glycosidic substances isolated from fresh bud scales were found to have linear association with smut resistance. Lyold and Pillay (1980) identified flavonoids as inhibitors of teliospore germination and established a relationship between smut sensitivity to smut and polyamine conjugation. Infection of buds from both sensitive and resistant cultivars of sugarcane with teliospores of *S. scitamineum* lead to remarkable increase of both free and conjugated polyamines. Conjugation of polyamines to phenolics has often been described as a defence mechanism against infection of several higher plants by viruses and fungi. Conjugation mainly affects tyramine conjugated to ferulic and hydroxycinnamic acids (Fleurence & Negrel, 1989) or spermidine and spermine conjugated to hydroxycinnamic acids (Hedberg *et al*., 1996). Lloyd and Naidoo (1983) proposed that phenolics are produced as a linear response of resistance acquisition against smut infection, it can be hypothesized that the conjugation of polyamines to phenolics can nullify the microbicidal action of these compounds. A negative relationship between glycosidic substance content in bud scale and resistance of sugarcane varieties to smut was observed, indicating that the glycosidic substance in bud scale might be a chemical mechanism of resistance against the infection of *Ustilago scitaminea.*

The level of different polyamines and the possible conjugation to phenolics in mature organs of *S. scitamineum*-infected and non-infected sugarcane plants has been suggested to be correlated with smut susceptibility, indicating that polyamine conjugation to phenolics may act as a mechanism of resistance or defense against this disease. Legaz *et al*. (1998) attempted to study the relationship between the sensitivity of resistance to smut with the accumulation of free or conjugated polyamines in sugarcane tissues, and observed that infectivity and development of fungal mycelium in sensitive buds could be clearly correlated with a dramatic increase of both SH and PH-spermidine and spermine.

Rodriguez *et al*. (2001) hypothesized key role of the oxidative burst on the early sugarcane response against the *S. scitamineum* infection. Results suggested that ethylene could be inducing sugarcane transcripts related to auxins and defense proteins. Xu *et al.* (1994) reported that infection by *Ustilago scitaminea* resulted in increase in peroxidase (POD) and invertase activity in both resistant and susceptible sugarcane plants. The results suggested that POD activity could be used as an index for smut resistance in sugarcane. PAL, TAL, CoA-ligase specific activities and chlorogenic acid, total flavone contents were measured in sugarcane varieties with different resistance to smut after inoculation with *Ustilago scitaminea* Syd.. PAL, TAL, CoA-ligase activities of highly resistant varieties were higher and maintained longer time than those of highly susceptible ones. At the same time, the accumulation of chlorogenic acid, total flavone contents in highly resistant varieties was not only earlier, but also quantitatively higher. Therefore, the results suggested that strengthening of phenylpropanoid metabolism induced by *Ustilago scitaminea* might be an important aspect of sugarcane post-infectional resistant mechanism to smut. The increase of activities of POD and acid invertase was also observed in leaves of sugarcane infected by sugarcane chlorotic streak virus (Wang *et al.,* 1995). Singh *et al*., (2002) observed an increase in the ascorbic acid content in leaf, bud, apical meristem, lateral shoots as well as in juice of smut affected stalks in two smut susceptible varieties. It was presumed that the enhancement in the ascorbic content in smut affected stalks might be due to the production of ascorbic acid accelerating enzymes by the pathogen or by the interaction of host-parasite.

The role of sugarcane glycoproteins in the resistance of sugarcane to smut was examined by many researchers. Sugarcane produces two different pools of glycoproteins containing a heterofructan as glycidic moiety and tentatively described as high molecular mass (HMMG) and mid-molecular mass (MMMG) glycoproteins (Legaz *et al*. 2005). Analysis of both HMMG and MMMG by capillary electrophoresis revealed that MMMG fraction contains two cationic and four anionic components, whereas only one cationic and four anionic proteins are separated from the HMMG fraction (Legaz *et al*. 1998). These glycoproteins affected polarization of the cytoplasm during spore germination, impaired germ tube protrusion and germination of the spores ultimately. These could be considered as factors contributing to smut resistance (Martınez *et al*. 2000). As their amount increases after infection with smut teliospores in resistant, but decreases in susceptible varieties after infection with smut teliospores. Fontaniella *et al*., (2002) ascertained the role of these glycoproteins in sugarcane smut resistance and recorded that Methyl jasmonate did not produce an elicitation response for glycoprotein synthesis in sugarcane. On the contrary, salicylic acid, secreted by germinating spores of *S. scitamineum* acted as an elicitor of glycoprotein production, and the elicitation process could be experimentally simulated by using this compound instead of spore inoculation. However, the quantitative response of sugarcane stalks to the infection in order to produce defence glycoproteins is higher than that obtained by infiltration of salicylic acid in plant tissues. The results opened up the possibility of the secretion of a co-elicitor, other than salicylic acid and unidentified as yet, seems to be required for the complete response. It has been proposed that the inhibition of teliospore germination constitutes a defence mechanism involved in the general pattern of the resistance of sugarcane to the smut pathogen.

Millanes *et al*. (2005) examined the role of sugarcane glycoproteins in regulating the cell polarity of *S. scitamineum*. Smut teliospores were found to be able to change the pattern of glycoprotein production by sugarcane, thereby promoting the synthesis of different glycoproteins that activate polarization after binding to their cell wall ligand. The study further demonstrated that smut teliospores were able to change the metabolism of parenchymatous cells of resistant sugarcane cultivars by increasing glycoprotein production. The results proposed that inhibition of teliospore germination constitutes a defense mechanism involved in resistance of sugarcane to smut. Millanes *et al*., (2008) hypothesized that the inhibition of smut teliospores germination by sugarcane glycoproteins, HMMG and MMMG, could be specifically related to actin polymerization. High molecular mass elicitors (proteins or glycoproteins) were previously detected in *Colletotrichum falcatum* (Went) (Ramesh Sundar *et al*., 2002), but these types of compounds from smut mycelium did not show biological activity.

Inoculation with the smut pathogen produced new phenolics, that increased the level of Hydroxy cinnamic acids (HCA) and their derivatives to enhance the synthesis of lignin and strengthening of the cell wall in the sugarcane cultivar resistant to *S. scitamineum*. de Armas *et al*., (2007) observed that the sensitivity or resistance of sugarcane to smut can be related to changes in the levels of free phenolic compounds, and phenylalanine ammonia-lyase (PAL) and peroxidase (POD) activities in the leaves. Elicitors from *S. scitamineum* enhanced the activity of PAL and consequently increased the levels of hydroxycinnamic and hydroxybenzoic acids. However, a decrease in the amount of free hydroxycinnamic acids was found, when the highest PAL activity was reached. It was concluded that monitoring changes in leaf phenolic compound concentrations, PAL and POD activities in response to

soluble elicitors extracted from *S. scitamineum* mycelium could afford reliable analyses of the resistance of sugarcane to smut. A resistant cultivar needs to maintain a high level of PAL activity without accumulation of free hydroxycinnamic acids. Increase in POD activity is important in the defence mechanism, but it is not a determinant for the defence mechanism. This model was proposed for the screening of smut resistance levels of different sugarcane cultivars and it would help breeding programs to characterise promising clones. Further it was concluded, that it is possible to say that the metabolism of phenylpropanoids seems to be directly related with resistance to smut.

Santiago *et al*., (2008) identified smut-elicitor fractions as resolved by capillary electrophoresis. Those inducing the highest biological activity corresponded to negatively charged proteins, peptides or glycopeptides of medium molecular mass. These compounds enhanced the accumulation of free phenolics, mainly hydroxycinamic acids, by activation of PAL in the resistant cultivar, and hydroxybenzoic acids in the susceptible cultivar. Another important difference in the resistant cultivar was the enhancement of POD-an enzyme that uses free phenolics as substrates for the activation of important mechanisms of resistance of sugarcane leaves to the fungal pathogen. Santiago *et al*., (2010) further correlated changes in the levels of phenolics substances, induced by a smut elicitor, which resulted in increase in thickness of the lignified cell walls and thus could contribute as a possible mechanical defense response to the potential entry of the smut pathogen. It was hypothesized that lignin deposition in supporting tissues might be indicative for biochemical and structural resistance responses in sugarcane.

7.2 Molecular markers for smut resistance

In order to understand the mechanism behind disease resistance in sugarcane, recent studies include molecular approaches involving Genomics and Proteomics tools. With the advent of such sophisticated tools of biotechnology, it has now become possible to gain better understanding on sugarcane-pathogen interaction. The processes that determine the outcome of an interaction between a microbial pathogen and a host plant are complex. Understanding the molecular details of these interactions, such as the pathogen genes required for infection, effective host defense responses and mechanisms by which host and pathogen signaling networks are regulated, might be utilized to design new plant protection strategies. A major limitation, however, is the poor availability of genetic tools in sugarcane because of the genomic complexity due to its polyploidy nature. Nevertheless, further characterization and functional analysis of the genes that are identified in the Sugarcane EST (SUCEST) program can lead to a more comprehensive understanding of sugarcane-pathogen interactions.

AFLP-based genetic mapping strategy by Raboin *et al*., (2001) focussed on a cross between cultivar R 570 (resistant) and cultivar MQ 76/53 (highly susceptible), which showed a segregation for smut resistance in a preliminary field trial. The findings established correlations between segregating markers and resistance to smut and discussed the possibility of identifying the different components involved in smut resistance and the interest of locus specific markers (SSR, resistance gene analogs, etc) to refine the genetic map. Thoakoane and Rutherford (2001) explored the possibility of isolating differentially expressed genes in sugarcane in response to challenge with the smut pathogen by using cDNA -AFLP. Sequence homology searches of isolated genic fragments have identified a

putative chitin receptor kinase, a Pto ser/thr protein kinase interactor, and an active gypsy type LTR retro-transposon expressed differentially in the resistant variety in response to challenge. Sugarcane genes encoding proteins homologous to chitinases, as well as transcripts related to the pathways of both phenylpropanoids and flavonoids were shown to be involved in the sugarcane resistance after 7 days of *S. scitamineum* infection.

Sequence analysis of genes differentially expressed in response to challenge by smut has identified putative receptors involved in the signalling of resistance mechanisms, transcription factors, and enzymes involved in phenylpropanoid-flavonoid metabolism (Heinze *et al*., 2001). Two full-length thaumatin (PR5) antifungal protein coding sequences have been isolated and are available for use as transgenes. Constitutive expression of acidic thaumatin suggested the involvement of SA signalling in sugarcane buds, as does the presence of a putative SA inducible cell-wall bound receptor kinase.

Genes encoding NBS-LRR-like proteins, protein kinases, and proteins related to both auxin and ethylene pathways were found to contribute to stable resistance against the sugarcane smut pathogen (Borras *et al*. 2005). The studies by Butterfield *et al*., (2004) and Hidalgo *et al*., (2005) demonstrated that subtractive or differential display techniques could be used to identify genes, that are activated during biotic stress responses, such as those induced by pathogens, and allow the isolation of rare transcripts elicited as part of the plant's resistance response. Results of the Northern blot analysis indicated that mRNA levels of genes, that are homologous to four of those transcript-derived fragments (TDFs) were highly induced in resistant somaclones inoculated with *S. scitamineum*, while no or low expression was observed in the susceptible parental lines, thus confirming the differential expression pattern. The differential expression of a number of sugarcane genes upon inoculation with the sugarcane smut fungus *S. scitamineum* was affiliated with disease resistance, as it makes sense that they should have the potential to be developed into markers for resistance.

In sugarcane, the expression pattern of a putative ethylene receptor (SCER1) and two putative ERF transcription factors (SCERF1 and SCERF2) showed differential responses to interactions with pathogenic and beneficial microorganisms, which suggested that they might participate in specific ethylene signaling cascade(s), that can identify a beneficial or pathogenic interaction (Cavalcante *et al.* 2007). Que *et al*., (2008) attempted to isolate resistance gene analogs (RGAs) from sugarcane (*Saccharum officinarum* Roxb.) with primers targeting the conservative sequences of nucleotide-binding site (NBS). A full-length cDNA of cRGA1 (Accession number: EF155648), termed *SNLR* gene, was cloned and its expression profile under the treatment of *S. scitamineum*, SA and H_2O_2 was investigated by real-time RT-PCR (Accession number: EF155654). The results showed that *SNLR* gene could be to some extent influenced by *S. scitamineum* and SA, but not by H_2O_2. Based on the results of Que *et al*., (2008), it was hypothesized, that this might be due to the reason that the *NLR* gene does not occur *via.* an H_2O_2 dependent pathway or involves a different mechanism. Further work on the functional genomics part involving transgenic complementation, gene knock-out or other experiments would add more information to establish its function in smut resistance. Subsequent investigations by Que *et al*., (2009) indicated the presence of non-TIR-NBS-LRR type resistance genes only in the genome of sugarcane. The 11 RGAs, together with *RPS*2 and *Xa1*, were clustered into one group, and *N* and *L*6 were in another group. One RGA, termed *PIC* (EF059974), was validated through real-time PCR. The result showed that the expression of *PIC* gene was induced by *S. scitamineum* and salicylic acid,

but inhibited by hydrogen peroxide. The *PIC* gene had constitutive expressions in leaves, stalks, and roots of sugarcane, with the strongest expression in leaves, which has a proven correlation with resistance to several diseases in sugarcane.

Lao *et al*., (2008) established the involvement of major plant signaling pathways during the first 72 h of interactions between sugarcane and *S. scitamineum*. A differential expression study on the *Saccharum* spp.–*S. scitamineum* pathogenic interaction was undertaken involving a susceptible (Ja60-5) and a resistant (M31/45) genotypes. A total of 64 transcript-derived fragments (TDFs) were found to be differentially expressed by using cDNA-AFLP analysis, wherein a majority (67.2%) of the differential TDFs was found to be up-regulated in the resistant M31/45 cultivar. The plant response against *S. scitamineum* infection was complex; representing major genes involved in oxidative burst, defensive response, ethylene and auxins pathways during the first 72 h post-inoculation. Results of this study suggested that the genes involved in the oxidative burst and the lignin pathways are vital for the initial sugarcane defense against the *S. scitamineum* infection. Segregation studies of the differentially expressed genes in "R" and "S" sugarcane progenies may provide more insight into the genetic basis of smut resistance in sugarcane.

8. Quarantine

In Australia, Sugarcane smut was identified as a high-risk exotic disease in a pest risk analysis conducted, and a contingency plan to deal with incursions was prepared in 1997, since its first time report in Australia in July 1998. Quarantine regulations were enacted in Queensland and New South Wales to reduce the risk of spread by plant material or appliances. The ORIA cane growers cooperated by ploughing out heavily infested fields and had removed all susceptible cultivars by 2001. This has reduced the risk of wind-borne spread from the ORIA. Nearly 20% of the germplasm collections maintained at Thailand recorded smut incidence (Jaroenthai *et al*., (2007), which has resulted in the reduction of yield, CCS, and brix by 8–18%, 7–13% and 17–43%, respectively. Infection and severity of the smut disease normally increased in ratoon cane, because smut spores can spread with wind, rain and the pathogen can survive in dry soil for 2–3 months. However, level of infection and severity also depended on the resistance of each variety.

Magarey *et al*., (2008) highlighted the perceived threats due to diseases and insect pests to *Saccharum* germplasm in Australia and neighbouring countries. An Australian centre for International Agricultural Research (ACIAR) funded program on conservation of germplasm was implemented in Papua New Guinea, Indonesia and Northern Australia. Since these areas constitute the centre of diversity for various *Saccharum* spp. there was increasing threats to the germplasm observed. Smut was perceived as one of the possible threats in Australia, as there was regular exchange of germplasm from neighbouring countries. In view of the alarming situation, a concerted breeding program was initiated, in which more than 1500 Australian clones have been screened for smut resistance in Indonesia.

9. Management

Seed selection and selective rouging of infected clumps would assure a healthy crop. Periodical observations of the standing crop and removing the whips would considerably

reduce the amount of pathogen inoculum, thus preventing further build-up of the pathogen. Studies reported that smut teliospores lack dormancy and hence could not survive in soil or debris in the absence of buds. This prompted advocation of deep ploughing and irrigating the fields, which will allow germination of the teliospores and would eventually die off in the absence of buds.

9.1 Physical control

Various hot water treatments have been reported to be effective in controlling the smut pathogen residing in the planting setts. The loss of bud germination due to inappropriate temperature settings needs to be handled properly (Srinivasan & Rao, 1968). Hardening of setts prior to hot water treatment was observed to considerably improve upon the germination of the buds. The efficacy of moist hot air treatments have been reported by Misra *et al*., (1978). Gupta *et al*., (1978) reported production of thicker and heavier canes with an increased number of millable canes due to hot water treatment.

9.2 Chemical treatment

Vangaurd and Bayleton treatment inhibited smut development from systemically infected seed pieces (Comstock *et al*., 1983). It is a recommended practise to subject the planting setts to a hot water treatment @ 52°C for 30 min combined with a chemotherapy using 0.1% Triademiphon - Bayleton (Mameghmay, 1984). This treatment was found to completely eliminate the sett-borne infection of smut.

Wada *et al*, (1999) suggested effective strategies for the management of sugarcane smut, *viz*. pre-plant heat therapy of planting setts; pre-plant fungicidal dips of planting setts and screening of sugarcane clones for identification of resistant varieties. It was observed that these single strategy controls might not be adequate for many sugarcane pests and diseases including smut, thus opting for IPM strategy, which would be a viable and successful smut management strategy. The need for continuing tests of different fungicides with varying modes of action for smut control has been discussed by Wada (2003). The best disease control was obtained with pyroquilon at 4.0, carbendazim+maneb at 4.57 and metalaxyl+carboxin+furathiocarb at 9.9 g a.i. Kg^{-1}, respectively. The efficacy of pyroquilon and metalaxyl+carboxin+furathiocarb, which *hitherto* were used as a seed treatment in cereals, revealed the availability of alternative uses for them in smut control.

Joyce *et al*., (2008) attempted to utilise smut resistant varieties in genetic modification research programs leading to commercial GM crop development in Australia. Protocol optimization was done for selecting an efficient tissue culture medium to produce embryogenic calli with high transformation efficiency.

10. Conclusion and future perspective

Sugarcane smut continues to be a serious threat to sugarcane production in different countries. Integrated disease management strategy is the viable option in smut disease control, rather than resorting to a single method. Recommended phytosanitary practices like seed selection, roguing of infected clumps etc is the best possible way to reduce smut inocula levels. Research on identifying sources of smut resistance in the germplasm and

progenies needs to strengthened. Development of smut resistant varieties to the current pathotype of *S. scitamineum* with a focussed breeding program, combined with clean cultivation practices would lead to successful management of smut disease in sugarcane. An understanding of the existing race picture of the pathogen is a pre-requisite for disease management, which could be accomplished by harnessing the tools of biotechnology. Recent literature attempts at throwing more light on understanding the biochemical and molecular basis of smut resistance in sugarcane. Though limited information is available regarding the sources of resistance, molecular tools are now available to identify suitable markers that can be relied upon for supporting the conventional breeding approaches. Similarly molecular diagnostic tools should be developed for a rapid and precise detection of the smut pathogen in seed cane. This supplemented with a strict quarantine regulations would prevent introduction of the disease into a new region and ensure supply of disease free seed material for planting. Information availability on the epidemiology of smut disease is very limited and more emphasis should be given to study the influence of critical weather parameters on smut severity, as this would lead to a better understanding on the impact of climate change on this important disease of sugarcane. Also efficient decision-support systems need to be developed for smut disease forecast, thus will result in the development of precise forewarning systems of a possible outbreak of the disease. In addition to the existing control measures, novel strategies should be thought of to explore the possibility of inducing systemic resistance against the smut pathogen. Further with the identification of candidate defense genes, development of transgenic sugarcane with built-in resistance to smut is to be looked into for the future.

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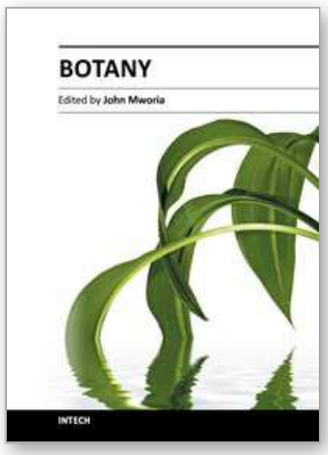

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This book is devoted to botany and covers topical issues in this diverse area of study. The contributions are designed for researchers, graduate students and professionals. The book also presents reviews of current issues in plant-environment interactions making it useful to environmental scientists as well. The book is organized in three sections. The first section includes contributions on responses to flood stress, tolerance to drought and desiccation, phytotoxicity to Chromium and Lead; the second has aspects of economic botany including a review of Smut disease in sugarcane and properties of plant extract used Tassaboount date juice; the last covers topical issues on morphogenesis and genetics on cotton fiber special cell, secretory glands Asphodelus aestivus flower ,pollen tube growth in Leucojum aestivum , morphological studies of Ardisia crenata complex, and hybrid lethality in the Genus Nicotiana.

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